Detecting nanoscale vibrations as signature of life

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The existence of life in extreme conditions, in particular in extraterrestrial environments, is certainly one of the most intriguing scientific questions of our time. In this report, we demonstrate the use of an innovative nanoscale motion sensor in life-searching experiments in Earth-bound and interplanetary missions. This technique exploits the sensitivity of nanomechanical oscillators to transduce the small fluctuations that characterize living systems. The intensity of such movements is an indication of the viability of living specimens and conveys information related to their metabolic activity. Here, we show that the nanomotion detector can assess the viability of a vast range of biological specimens and that it could be the perfect complement to conventional chemical life-detection assays. Indeed, by combining chemical and dynamical measurements, we could achieve an unprecedented depth in the characterization of life in extreme and extraterrestrial environments.

nanomechanical sensors | extraterrestrial life | nanoscale fluctuations | living specimens | nanomotion detector

The existence of life in extreme conditions, in particular in extraterrestrial environments, is certainly one of the most intriguing scientific questions of our time. Indeed, the work of many scientists and organizations is focused on the discovery and on the consequent study of extremophiles and extraterrestrial organisms. The direct research for these kinds of life forms is usually conducted by deploying robotic crafts. These man-made vessels contain a suite of scientific analytical instrumentation that is specifically conceived to trace life signatures contained in the geological record. For instance, the search for life in our solar system started in 1975 with the Viking program and continues today. Future missions are planned to explore the presence of life on satellites of the giant planets, such as Europa (Jupiter) or Titan and Enceladus (Saturn). The biological instrumentation that is included in these vessels is complex, but up to now, it is mainly devoted to the chemical detection of molecules involved in living metabolism, as we know it on Earth.

In this report, we show how a technique, the nanomotion detector, can be used in new life-searching instrumentation in Earth-bound and interplanetary missions. The technique exploits the sensitivity of nanomechanical sensors to transduce the small movements that characterize living systems. The intensity of such movements is an indication of the viability of the specimens and conveys information related to their metabolic activity. Here, we demonstrate that this simple technique can assess the viability of a vast range of biological specimens and that it could be the perfect complement to conventional chemical assays. Moreover, due to the simplicity of its working principle, a device based on this technology has negligible weight and requires very low electrical power, compared with other life-detector systems.

Nanomechanical oscillators are extremely sensitive devices that are commonly used to measure very small deflections and detect forces in the order of the piconewton. These sensors can be used to characterize living specimens and their metabolic cycles (1–4). For instance, recent studies have shown how cantilevers can investigate the activity of a cell's molecular motors (5), enzymes adsorbed on mica (6), or the particular vibrations of living *Saccharomyces cerevisiae* (7, 8). Overall, these extremely powerful and versatile sensors are capable of characterizing biological systems with unprecedented detail and time resolution and are nowadays used for several biological applications (9–16).

To extend the scope of the scientific problems that can be investigated with this tool, we have recently demonstrated how nanomechanical oscillators can be used to study nanometer-scale movements in biological specimens (17). In this pioneering work, we have exploited this nanomotion detector to monitor the viability of bacteria in the presence of different antibiotics and to assess rapidly (in less than 30 min) a complete bacterial antibiogram (18). Recently, other groups have used our technique to confirm our findings and to study the fluctuations induced by bacteria on a cantilever (19).

The working principle of the technique may be summarized as follows. A microfabricated atomic force microscopy (AFM) cantilever is inserted into an analysis chamber, and specimens are attached to its surface. The cantilever transduces the movements of the samples with a subnanometer resolution. The dynamic deflections of this sensor are detected and recorded using a laser-based transduction system. The time resolution and sensitivity of this system make it ideal to study living specimens at the nanoscale. It can be operated in air or in a liquid environment; in this latter case, the living specimens can be exposed to triggering or inhibiting chemicals to characterize their response to the stimuli (Fig. 1). Furthermore, the small size, relative simplicity, and versatility of the setup open the way to its parallelization: several single detectors can be used at the same time to form a complex laboratory-on-a-chip system capable of detecting and characterizing unknown living organisms.

Here, we demonstrate the use of this sensor to study a large range of biological specimens, ranging from prokaryotic to eukaryotic organisms. The motion and metabolic state of all

Significance

The quest to find life in extreme and extraterrestrial environments is exciting and touches many research fields. One of the common signatures of life is movement: Even small microorganisms vibrate in response to their metabolic activity. Thus, we have devised a nanomotion detector to study these fluctuations and to associate them to the metabolic activity of the specimens. This technique does not measure the chemical response of life, which would require prior knowledge of the metabolic pathways involved. Instead, it monitors the physical manifestation of any kind of metabolic activity the microorganisms might have. Here, we show how this nanomotion detector can study any living system, paving the way to a complementary approach to the study of life in extreme environments.

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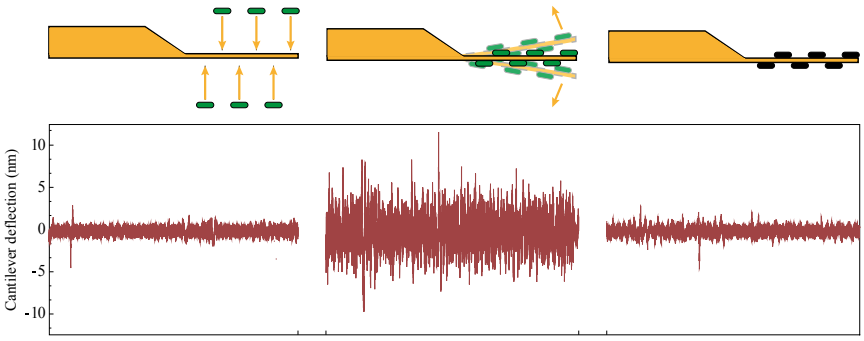

Fig. 1. Detailed depiction of a nanomotion detection experiment. Before the attachment of the living specimens to the sensor, the fluctuations are small (*Left*). When the specimens are immobilized on the sensor, its fluctuations increase (*Center*). Finally, if the microorganisms are killed, through a chemical or physical agent, the sensor reverts to small fluctuations (*Right*).

these systems were artificially activated and repressed by exposing the samples to appropriate chemical components, to demonstrate that the detection system can be used as a simple, extremely sensitive, and weight-efficient "life detector." In each experiment, we immobilized living samples on the cantilever sensor, and we monitored the evolution of its fluctuations over time. We investigated the viability of a wide range of single cellular living organisms. Among bacteria and yeasts, we studied Gram-negative motile *Escherichia coli* (Fig. 2*A*), Gram-positive nonmotile *Staphylococcus aureus* (Fig. 2*B*), and *Candida albicans* yeast cells (Fig. 2*C*). Measurements were also carried out on eukaryotic cells, such as mouse osteoblasts (MC3T3-E1) (Fig. 3*A*), human neuroblasts (M17) (Fig. 3*B*), and plant cells (*Arabidopsis thaliana*) (Fig. 3*C*). A detailed description of the experimental protocols and of the individual experiments is presented in *SI Text*.

In all cases, the presence of the living systems on the cantilever surface produced an increase in the amplitude of the measured fluctuations. The experimental evidence suggests that these fluctuations reflected the metabolic state of the microbes or of the cells. Indeed, upon the injection of nutrients into the analysis chamber, the amplitude of the oscillations increased whereas the exposure to inhibiting agents stopped the movements of the cantilever, indicating that the chemical affected the specimens. To better visualize the macroscopic effects of the stimuli, some experiments were performed while imaging the cantilever with a conventional optical microscope (examples for each experiment are shown in the respective panels in Fig. 2 for microorganisms and Fig. 3 for cells whereas Movies S1–S3 show a time-lapse reconstruction of the microscopic movements of the eukaryotes throughout an entire experiment). Comparing the optical images with the nanomotion data gives insight into the

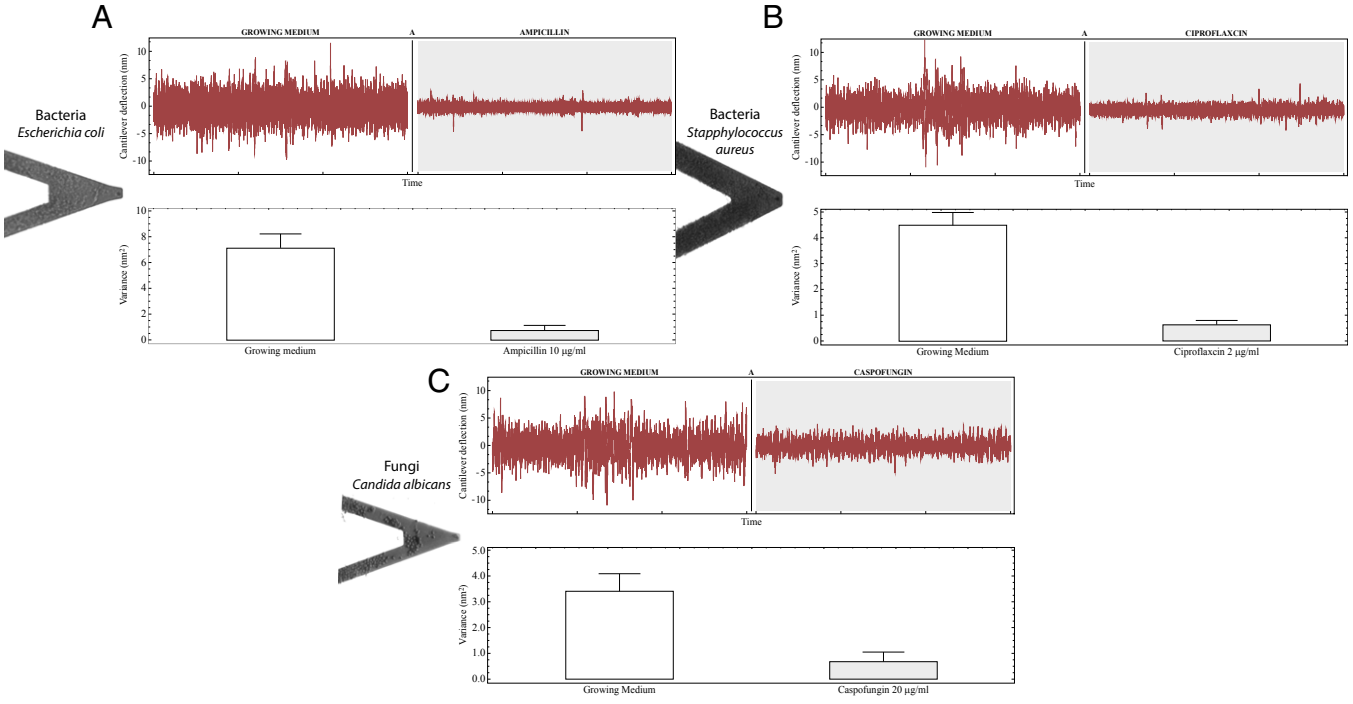

Fig. 2. Experiments on bacteria and yeasts. (*A*) Experiment involving *E. coli*. Two typical 10-min segments of the sensor's fluctuations are shown: the living bacteria induced a large fluctuation of the sensor whereas, 15 min after injection of a bactericidal dose of ampicillin, the fluctuations were largely reduced. (*B*) Experiment involving *S. aureus* exposed to a bactericidal dose of ciproflaxcin. Here, we present two typical 10-min segments of the fluctuations in buffer and after exposure to the antibiotic. (*C*) Experiment involving *C. albicans* exposed to a fungicidal dose of caspofungin. We show typical 2-min segments of the sensor's fluctuations before and after exposure to the antifungal.

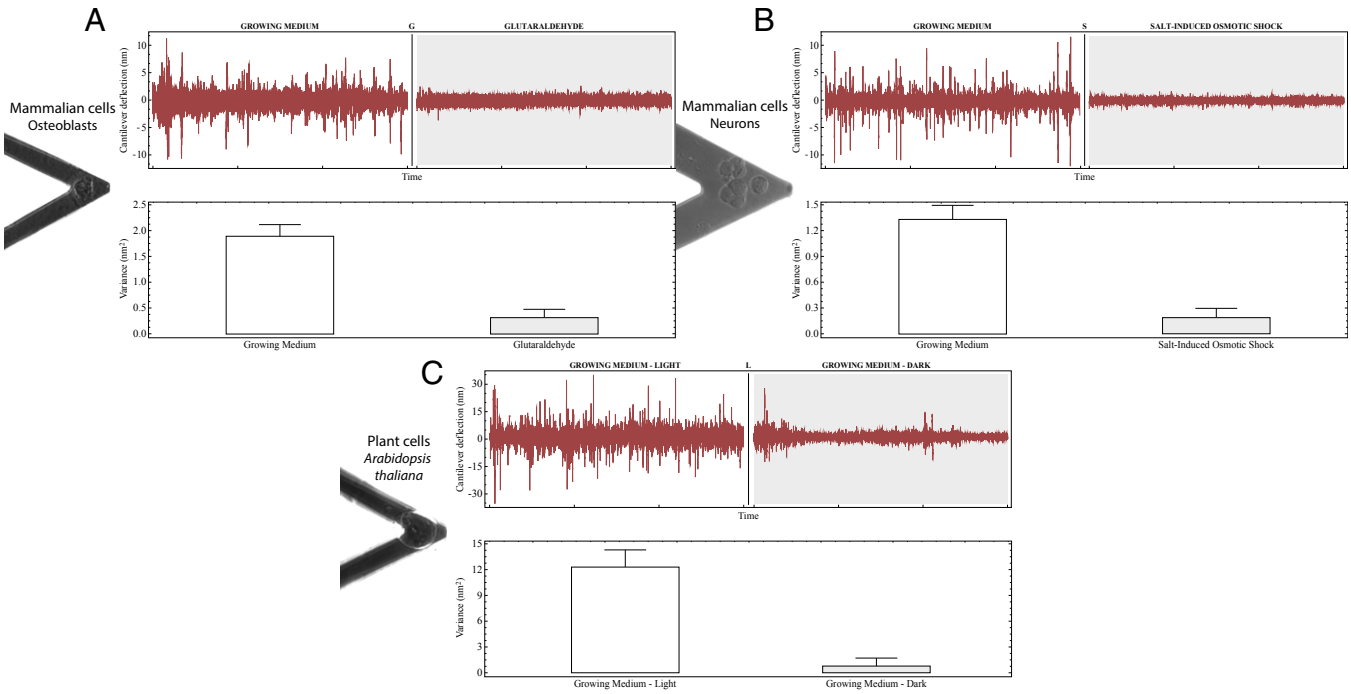

Fig. 3. Experiments on eukaryotic cells. (*A*) Experiment on osteoblast cells. Two typical 10-min segments of the sensor's fluctuations are shown: a cell was attached to the sensor, and its movements induced large fluctuations. When the cell was killed through chemical fixation, the fluctuations were reduced. (*B*) Experiment involving neuroblast cells. Here, we present two typical 10-min segments of the fluctuations in buffer and after inducing the death of the cells through osmotic shock. (*C*) Experiment involving *A. thaliana* cells. We show typical 2-min segments of the sensor's fluctuations while keeping the cell illuminated and after a prolonged period in a dark environment, which induced the death of the cell. We monitored all three experiments using optical images, as shown in Movies S1–S3.

origin of some fluctuation structures. Moreover, the optical images confirmed that the injection of different media, as well as chemical or physical stimuli, did not cause detachment or macroscopic displacement of the cells over the cantilever.

Our previous results show that the fluctuations convey information on the overall metabolism of the specimens and that they are much more than a mere viability test (18, 20). Thus, we performed some experiments specifically designed to understand the origin of the measured fluctuations. We have focused on the pathways involved in the internal motion or in the propulsion of the different specimens and studied the variations of the fluctuations upon their chemical deactivation. In one set of experiments, we studied the effect of the movement of *E. coli*'s flagellum on the overall fluctuation of the sensor. We performed this experiment by exposing the bacteria to a high concentration of glucose solution, which inhibits the motion and, in some cases, even the synthesis of the flagellum (21). These experiments are described in detail in *SI Text* and show that the exposure to glucose caused, at first, an increase in the overall fluctuations, probably induced by the digestion of the added energy source. This first increase was followed by a reduction of the sensor's amplitude, which could be the fingerprint of the inhibition of the flagellum (Figs. S1 and S2).

In other experiments, we investigated the role on the resulting fluctuations of different cytoskeleton components of mammalian cells. Osteoblasts were treated with chemical agents that induced the depolymerization of either the actin (Fig. S3 and Movie S4) or the tubulin cytoskeletal networks (Fig. S4 and Movie S5).

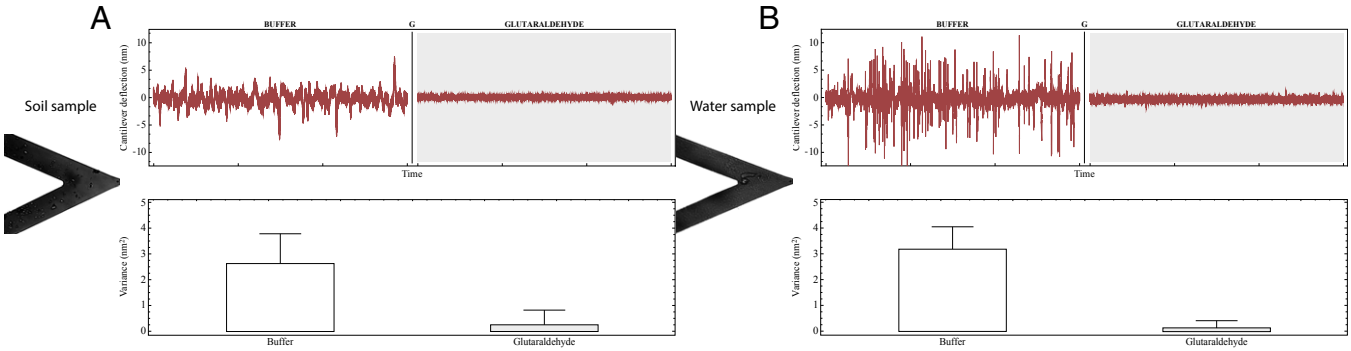

Fig. 4. Experiments involving soil and water samples. (*A*) Typical 4-min segments of the sensor's fluctuations induced by the presence of microorganisms from the soil sample are shown. The viability of the specimens caused large fluctuations, and, when glutaraldehyde was introduced to kill all microorganisms, the fluctuations returned to low levels. (*B*) The living systems present in the water samples induced an increase in the amplitude of the movements of the sensor. After chemical fixation, the movements were reduced.

These experiments, recounted in *SI Text*, show that the high temporal resolution of the nanomotion detector evidences two different subgroups of fluctuations, which could represent the signature of the movements of the actin and of the tubulin networks. Specifically, large fluctuations of the sensor can be associated with movements inside the actin network whereas less intense but more frequent fluctuations can be attributed to the tubulin network. Naturally, some more studies will be needed to understand fully all of the components of the signal produced by living systems. In fact, we have recently demonstrated that even small conformational changes in proteins can induce fluctuations of the cantilever sensor (22). Thus, we can conclude that nanoscale movement is a universal signature of life and that every living system exhibits a large and diverse variety of movements that are related directly to their viability.

One remarkable peculiarity of the nanomotion detector is that it does not need a complete characterization of the specimens under investigation to detect their presence and viability. In fact, we were able to perform completely blind experiments in which the samples were originated from uncontrolled sources and completely unknown. We collected some dry soil from the fields and some water from the river Sorge, near our university campus. A first simple optical microscopy investigation evidenced the presence of different kinds of microorganisms, including bacteria and small unicellular species. We performed a nanomotion detection investigation: just like in the other controlled experiments, we diluted the samples in the analysis chamber and immobilized them on the cantilevers. Next, we measured the fluctuations of the sensors in a buffer solution. The fluctuations of the cantilever are shown in Fig. 4 and confirm that the soil (Fig. 4*A*) and water samples (Fig. 4*B*) contain living specimens. Their movements on the sensor's surface caused large fluctuations of the cantilever whereas, by chemically inducing their death, these fluctuations were greatly reduced.

Remarkably, in each of the experiments described in this work, we needed just a few tens of minutes to determine the presence of viable microorganisms. Moreover, a very small number of living specimens diluted in just a few microliters of solution were sufficient to perform the experiments.

These results demonstrate that this technique can efficiently identify the activity produced by a wide range of living organisms that inhabit Earth and that, in some cases, the collected data can help identifying the specific signature of particular cellular movements. Even if the sample under investigation is not characterized, the nanomotion detector can rapidly and reliably deliver information on its viability. In fact, whereas most of the conventional life detectors currently used in biology and astrobiology look for the chemical signatures of life, this technique is focused on monitoring a physical quantity: the nanometer-scale movement. This technique offers a complementary point of view in the search for life in extreme habitats. For instance, it could allow the detection of systems with novel and unexpected metabolic pathways. Indeed, by combining chemical and dynamical measurements, we could achieve an unprecedented depth in the characterization of life in extreme and extraterrestrial environments.

Furthermore, these results will be useful to define the physical and chemical limits for life on Earth and to understand the underlying biophysical characteristics of life that set these limits (23). Finally, the simplicity of its working principle and the possibility to miniaturize and parallelize the apparatus make this device a good candidate to be embarked in future life-seeking spaceships.

Methods

To perform the experiments, we used commercial silicon nitride, microfabricated 200-μm-long cantilevers, with a nominal spring constant of 0.06 N/m (DNP-10 Bruker). The oscillations of this lever were detected through the deflection of the laser beam of a commercial AFM; for all of the presented experiments, we used a Nanowizard III (JPK). The data analysis was completed with custom software written in LabView (National Instruments).

The typical setup of the experiments is described in detail in our previous works (18, 20). Briefly, a typical experiment started by depositing the specimen of interest onto the sensor, which was preliminarily functionalized with a linker molecule. Depending on the particular system under investigation, we chose the functionalization that had demonstrated the best immobilization efficiency. For bacteria, yeast, and plant cells, we chose glutaraldehyde; for neuron cells, we functionalized the sensor with poly-L-lysine; and osteoblasts required a fibronectin coating (see the *SI Text* for a detailed description of the cantilever preparation for each experiment). The fluctuations of the cantilever were recorded in nourishing media and were used to define the viability of the living organisms. Subsequently, the cantilever was exposed to chemical or physical conditions that brought the living specimens to death, resulting in reduced fluctuations of the sensor. To evaluate the experimental results, the fluctuations of the cantilever were statistically analyzed by calculating their variance.

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